

iGEM TU/e 2014

Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2014.igem.org/Team:TU_Eindhoven

Date

4 August 2014

Protein expression

General protocol for incorporation of the unnatural amino acid

Table of contents

Title	1	Growth of small bacterial culture	3
Protein expression	2	Stock solution p-Azido-L-Phenylalanine (pAzF)	3
	3	Protein expression	3

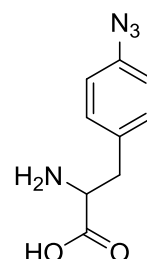
1 Growth of small bacterial culture

- Work near the Bunsen burner flame
- Fill a 15 mL sterile culture tube with 5 mL of 2YT
- Add 5 μ L kanamycin (30 μ g/mL) and 5 μ L chloramphenicol (25 μ g/mL) to the culture
- Pick cells from the glycerol stock using a sterile pipette tip and throw the pipette tip in the culture tube
- Grow the bacteria overnight at 37°C and 250 rpm

2 Stock solution p-Azido-L-Phenylalanine (pAzF)

Solution 10 mM pAzF

- 2YT medium
 - DMSO: 5v%
 - pAzF: 10 mM
- shake at 4°C until all pAzF is soluted



Molecular Weight: 206.20
pAzF

3 Protein expression

- Transfer 100 μ L of the small culture to new 7 mL 2YT culture
- Add 7 μ L kanamycin (30 μ g/mL) and 7 μ L chloramphenicol (25 μ g/mL) to the culture
- Grow the bacteria at 37°C and 250 rpm
- Grow until OD=0.6, OD measurement first requires blank measurement with 2YT
- Wrap culture tube in aluminium foil
- For a 5 mL culture: add 5.62 μ L IPTG from the 1M stock, 56.24 μ L of arabinose from the 20% stock and 562.43 μ L of unnatural amino acid from the 10 mM stock to the culture wrapped in aluminum foil, in order to get end concentrations of 1 mM, 0.2% and 1 mM, respectively
- Perform protein expression for ~15 h at 25 °C and 250 rpm
- Spin down the cells for 15 min at 3,000 xg.
- Discard supernatant.
- Add 1 ml PBS-0.1%BSA and transfer to 1,5 mL Eppendorf microcentrifuge tube.
- Spin down the cells for 1 min at 13,000 xg.
- Discard supernatant.
- Add 1 ml PBS-0.1%BSA
- Fill 1 OD cuvette with 950 μ L dH₂O and 50 μ L PBS-0.1%BSA for blank OD measurement.
- Fill 1 OD cuvette with 950 μ L dH₂O and 50 μ L of culture sample (dilution of 20x)
- Measure OD, this has to be lower than 1, otherwise make a higher dilution.

- Calculate OD of culture sample. (If you made a dilution of 20X then the OD of the culture is 20X the OD of the dilution)
- Calculate amount of cells in culture
http://www.genomics.agilent.com/biocalculators/calcODBacterial.jsp?_requestid=826255
- Your answer of the previous step is in bacteria/mL
 Calculate amount of PBS-BSA 0,1% you have to add to obtain a final concentration of 10^7 cells/mL (in case of antibody titration) or 10^9 cells/mL (in case of reaction with DBCO). Use the following formula:

10^7 cells/mL	10^9 cells/mL
This will give your dilution: $Dilution = \frac{\left[\frac{Bacteria}{mL} \right]}{10^7}.$	This will give your dilution: $Dilution = \frac{\left[\frac{Bacteria}{mL} \right]}{10^9}.$
Amount of cells: <i>Amount of cells (mL) = 1/Dilution</i> because you will make 1 mL of 10^7 cells	Amount of cells: <i>Amount of cells (mL) = 1/Dilution</i> because you will make 1 mL of 10^9 cells
Amount of PBS-BSA 0,1%: <i>Amount of PBS – BSA 0,1% (mL)</i> $= 1 - \text{Amount of cells}$ will make 1 mL of 10^7 cells	Amount of PBS-BSA 0,1%: <i>Amount of PBS – BSA 0,1% (mL)</i> $= 1 - \text{Amount of cells}$ will make 1 mL of 10^9 cells

- Store at 4 °C with aluminum foil.